



## Technical Support Information Bulletin 1188

### Kaiser Test (Ninhydrin Test)

The **Kaiser Test** is a very sensitive test for primary amines. It is commonly utilized in solid phase peptide synthesis to determine if coupling reactions are complete. Ninhydrin reacts with the deprotected N-terminal amine group of the peptide-resin to produce an intense blue color. The Kaiser test is not reliable for detecting secondary amines. Thus, if the N-terminal amino acid is proline, pipercolic acid, or tetrahydroisoquinoline-3-carboxylic acid, another test such as the **Isatin Test** or the **Chloranil Test** is used.

#### Kaiser Test (Ninhydrin Test)<sup>1</sup>

##### Kaiser Test Solutions

###### Reagent A:

1. Dissolve 16.5 mg of KCN in 25 mL of distilled water.
2. Dilute 1.0 mL of above solution with 49 mL of pyridine (freshly distilled from ninhydrin).
3. Pour it into a small reagent bottle and label it "A".

###### Reagent B:

1. Dissolve 1.0 g of ninhydrin in 20 mL of n-butanol.
2. Pour into a small reagent bottle and label it as "B".

###### Reagent C:

1. Dissolve 40 g of phenol in 20 mL of n-butanol.
2. Pour it into a small reagent bottle and label it "C".

##### Kaiser Test Procedure:

1. Take 10-15 beads of resin in a test tube and label it S.
2. Take tube S and another empty tube designated R (reference)
3. To each tube add:  
2 to 3 drops of Reagent A  
2 to 3 drops of Reagent B  
2 to 3 drops of Reagent C
4. Heat both the tubes at 110°C for 5 minutes.
5. Compare the color with reference.

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<sup>1</sup> Wellings, D. A.; Atherton, E. "Methods in Enzymology Volume 289: Solid-Phase Peptide Synthesis" Ed. Fields, G. B. Academic Press, San Diego, 1997, p. 54.

**Colorless or faint blue color:** complete coupling, proceed with synthesis

**Dark blue solution but beads are colorless:** nearly complete coupling, extend coupling or cap unreacted chains

**Solution is light blue but beads are dark blue:** coupling incomplete, recouple

**Solution is intense blue and all beads are blue:** failed coupling, check amino acid, reagents, then recouple